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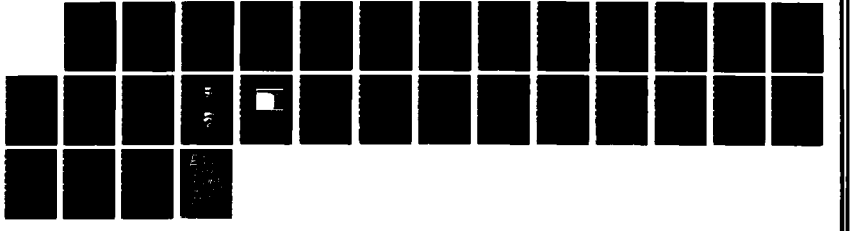

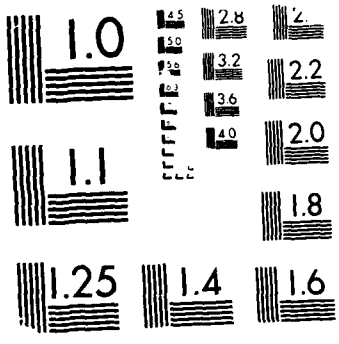

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TERATOGENICITY, MUTAGENICITY, AND EFFECTS OF GRADE 2 DIESEL FUEL ON REPRODUCTION IN A SINGLE GENERATION OF RATS

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Aberdeen Proving Ground, Maryland 21010-5423

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19 ABSTRACT (*Continue on reverse if necessary and identify by block number*)

Studies were conducted to investigate the potential teratogenicity of grade 2 diesel fuel (DF-2) smoke, its mutagenic potential to produce a dominant lethal mutation (DLM), and DF-2's effect on reproduction in Sprague-Dawley rats.

Pregnant dams were exposed to 2.34 ± 0.45 mg/liter of DF-2 smoke, 0.006 ± 0.006 mg/liter of tank exhaust for 1 hr and to noise from the tank's engine for 20-30 sec. One litter in the DF-2 smoke group had three fetuses with major, gross malformations and two with visceral abnormalities. The DF-2 smoke group also showed the greatest number of fetuses with sites of low bone ossification.

Males in the DLM study and the males and females in the single generation (SG) reproduction study were exposed to DF-2 smoke or exhaust for 15 or 60 min. Unexposed dams were (Continued on reverse)

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Lactation index	Smoke	
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Postnatal development	Survival index	

19. Abstract (Continued)

mated to DLM males from the 15-min exhaust group during the males' second week of post-exposure. These females had significantly more resorptions than the noise controls for the same mating period.

In the SG study, male pups in the 60-min DF-2 smoke group weighed significantly less than the noise control pups on day 1; this was also true for the female pups in the 60-min exhaust group on day 7. No difference in weight was apparent for any group by day 21. There was no difference in mating, fertility, delivery, or neonatal care across groups.

The major malformations observed in the teratology study occurred in rather isolated instances (i.e., in one litter in the DF-2 smoke exposed group and similarly in the exhaust and noise control groups). Retarded bone ossification was indicated in the DF-2 smoke group but without significantly lower body weights. Nothing indicates that DF-2 smoke caused fetal growth retardation. Observances in the DLM study were not strong enough to suspect a mutational effect, and the observations in the SG study gave no support for adverse effects on reproduction. Therefore, we conclude that DF-2 smoke does not cause teratogenic or DLM effects and does not adversely affect reproduction in the rat.

PREFACE

The work described in this report was authorized under Project No. 1L162622A554, Smoke Obscurants, Technical Area 4-E, Smoke Toxicology. This work was started in October 1979 and completed in July 1980. The experimental data are contained in laboratory notebook 10009.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the committee on Revision of the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

The use of trade names or manufacturers' names in this report does not constitute an official endorsement of any commercial products. This report may not be cited for purposes of advertisement.

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

TERATOGENICITY, MUTAGENICITY, AND EFFECTS OF GRADE 2 DIESEL FUEL ON REPRODUCTION IN A SINGLE GENERATION OF RATS

1. INTRODUCTION

Grade 2 diesel fuel (DF-2) is one of the materials being considered by the Department of the Army to generate smoke screens for field combat obscuration. The screen is to be produced by injecting DF-2 into the hot manifold of the vehicle being screened. Troops may be exposed to the smoke during operations; therefore, it is necessary to discern the smoke's potential danger to humans from inhalation. Studies by Callahan and co-workers, designed to elucidate the general toxicity of DF-2 smoke, showed only hypoactivity in rats exposed to the smoke for 60 min/day for 13 weeks.[1] The subject of this report is the effect of inhaled DF-2 on fetal development and reproductive processes using the rat as a model. These studies were initiated to determine whether teratogenic and/or dominant lethal mutational (DLM) effects or effects on reproduction could be elicited in a single generation (SG) of rats exposed to DF-2 smoke.

The chamber concentrations used were based on the expected field level.

2. MATERIALS AND METHODS

2.1 Materials.

2.1.1 Animals.

Sustained barrier, pathogen-free, random-bred colony rats, Sprague/Dawley-Wistar (SDXWI) Descendants, were obtained from the Veterinary Medicine Division of the U.S. Army Medical Research Institute for Chemical Defense (USAMIRCD), Aberdeen Proving Ground, Maryland.

2.1.2 Housing.

The rats were housed in polycarbonate plastic holding cages in an air conditioned building. The cages were 19 in. by 10.5 in. by 8.5 in. [model 18730 (Laboratory Products, Incorporated, Garfield, NJ)]. San-I-Cel bedding (Paxton Processing Company, Incorporated, Laurel Farm, White House Station, NJ) was used in the cages. The light/dark, manually controlled cycle was 12/12 hr.

2.1.3 Food.

The animals were fed Wayne Mouse and Rat Diet (Allied Mills, Incorporated, Chicago, IL), and tap water was available in nalgene polypropylene bottles (J & E Berge, Incorporated, South Plainfield, NJ).

2.1.4 Chemical and Emission Conditions.

The source, composition, and chamber monitoring techniques for DF-2 are explained by Callahan and co-workers.[1]

Smoke was produced by injecting DF-2 into the hot manifold of an M60A1 Tank. The exposure dosages were controlled by varying the length of time that the smoke was fed through a flexible pipe from the tank manifold into the chamber. Because the smoke was mixed with tank exhaust, the effect of exposure to exhaust fumes was also studied. The exposures were accompanied by the noise from the tank's engine; therefore, the control group for these studies was a noise control. For the noise control group, the engine was operated for 20-30 sec, which was the same length of time that the engine was operated during the exhaust and smoke groups' exposures. The exposures for the study follow:

- 60 min of chamber noise control
- 15 min of exhaust
- 60 min of exhaust
- 15 min of DF-2 smoke*
- 60 min of DF-2 smoke*

The chamber concentration of DF-2 smoke was 2.34 $\pm$ 0.45 mg/liter for both the 15- and 60-min periods. The concentration for exhaust was 0.006 $\pm$ 0.006 mg/liter for both periods. Concentrations were determined by using liquid chromatography to analyze chamber air samples for hydrocarbons.

2.1.5 Exposure Chamber.

The exposure chamber was a 20,000-liter, cylindrically shaped, steel chamber. The cages used for exposure were made of perforated stainless steel, had 1/2-in.^{2} holes, and were divided into 10 compartments. Each compartment measured 9 in. by 6 in. by 5.5 in. The cages were placed on holding racks inside the chamber.

The monthly means of the chamber temperature and relative humidity, measured at the end of each exposure, did not vary greatly across groups. The values for each month are shown in Tables 1 and 2.

2.2 Methods.

The studies followed the procedures recommended by the Environmental Protection Agency.[2]

All exposures were scheduled to cover specific events in the reproductive cycle of the rat. In the DLM and SG studies, male rats were exposed to the DF-2 smoke for 10 weeks, covering one complete cycle of spermatogenesis.[3] The exposures occurred before mating. DML males were mated with two sets of 12-week old, unexposed virgin females.

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*All DF-2 smoke exposures included exhaust.

Table 1. Monthly Means of Chamber Temperature (°F) Measured at the End of Each Exposure*

Month	Noise control (60 min)	Exhaust (15 min)	DF-2/Exhaust (15 min)	Exhaust (60 min)	DF-2/Exhaust (60 min)
August	78.0 ± 4.0	79.1 ± 3.0	78.3 ± 4.1	80.2 ± 5.1	77.8 ± 4.4
September	74.4 ± 4.6	74.5 ± 3.9	72.9 ± 4.2	73.7 ± 4.6	72.6 ± 4.1
October	70.2 ± 6.0	70.6 ± 5.0	70.4 ± 5.3	71.1 ± 5.2	70.2 ± 6.1
November	71.0 ± 2.5	71.1 ± 2.5	71.6 ± 3.4	72.0 ± 3.0	71.5 ± 3.6
December	69.6 ± 3.6	69.9 ± 2.0	70.4 ± 2.0	71.3 ± 1.9	71.2 ± 2.2

*Values extracted from Reference 1.

Table 2. Monthly Means of Chamber Relative Humidity Measured at the End of Each Exposure*

Month	Noise control (60 min)	Exhaust (15 min)	DF-2/Exhaust (15 min)	Exhaust (60 min)	DF-2/Exhaust (60 min)
August	82.0 ± 4.3	87.8 ± 4.0	91.0 ± 3.3	90.4 ± 4.4	87.8 ± 3.7
September	81.7 ± 6.8	80.6 ± 7.6	82.1 ± 10.0	80.8 ± 8.1	81.1 ± 9.2
October	68.2 ± 15.5	65.0 ± 13.8	63.6 ± 15.2	64.8 ± 14.9	62.9 ± 14.9
November	59.0 ± 8.0	58.6 ± 7.6	57.4 ± 8.4	58.0 ± 8.6	58.6 ± 9.4
December	46.7 ± 3.5	47.2 ± 2.7	47.2 ± 2.4	47.4 ± 2.6	47.3 ± 2.5

*Values extracted from Reference 1.

The first set mated during the first week after exposure and the second set during the second week after exposure. SG males were mated with 12-week old, virgin females that had been exposed at the same levels as their male mates for 3 weeks that coincided with the last 3 weeks of their mates' exposures. Their 3-week exposures covered 4-5 estrus cycles for the females.[4] In the teratology study, pregnant females were exposed to the DF-2 smoke from day 6 to day 15 of gestation (period of organogenesis).[5]

To preclude having fewer than the optimal number of animals in any group as a result of spontaneous or accidental deaths at the end of exposures, two excess males and four females were included in each group during exposures. At the end of the exposure periods, groups that still had excess animals were culled by random selection.

2.2.1 Fetal Toxicity and Teratogenicity.

One hundred fifty 12-week-old virgin females were mated to seventy-five 12-week old males (two females were mated to each male). The females were checked for insemination every morning; insemination was determined by the presence of sperm in the vaginal wash. Physiological saline was used as the wash fluid. The day sperm was found in the wash was considered day 0 of gestation.[6]

Females with sperm in their vaginal washings were assigned (two at a time) to the control group, the exhaust group, or the DF-2 smoke group until there were 22 sperm-positive females in the 1-hr control, exhaust, and DF-2 smoke groups. Male rats from these matings were euthanized and incinerated.

The exposure period for the sperm-positive females began on the calculated 6th day of gestation and continued through the 15th day of gestation. On the 20th day of gestation, 20 dams each from the control, exhaust, and DF-2 smoke groups were euthanized. Each dam was weighed, and a laparotomy was performed to expose the uterus. The viable and nonviable implants were counted, noting the positions of nonviable fetuses. The fetuses were delivered by caesarean section, grossly examined to check for abnormalities, sexed, weighed, and tagged. One-half of each litter was placed in Bouin's solution for subsequent serial sectioning to examine the viscera using Wilson's method;[5] the other half of the litter was placed in 95% ethanol to harden for subsequent staining and examination of the skeletal systems. The initial data recorded were the total number of implantation sites in each uterine horn, the number of viable fetuses, the number of nonviable fetuses (resorption sites), and any gross abnormalities. More detailed data on each pup was recorded during visceral or skeletal examination.

2.2.2 Dominant Lethal Mutation Screen.

Twelve proven males were randomly assigned to each of the study's five exposure groups. Each group was exposed 5 days/week for 10 weeks. During the week following the exposure period, each of 10 randomly selected males in each group was housed for 5 days with two 12-week-old virgin females for mating. After 5 days, these females were removed, and the males rested for two days. A second pair of virgin females was introduced for the second postexposure mating and were removed after 5 days. They were euthanized

11 days after their separations from the males. These females were necropsied to ascertain pregnancy and to record the number of viable fetuses, nonviable fetuses, and corpora lutea. Data in these categories were analyzed using Student's "t" test, the Freeman-Tukey Arc Sine transformation followed by Student's "t" test, and Chi-square analysis, respectively.[7]

2.2.3 Reproduction in a Single Generation.

Groups of 12 proven male rats were exposed to each exposure condition for 10 weeks. Twelve-week-old virgin female rats in groups of 24 were exposed similarly for 3 weeks, covering 4-5 estrus cycles. These females' exposures coincided with the last 3 weeks of the 10 weeks of exposure for the males. During the week following the end of the males' exposure periods, the animals were cocaged (two females per male) for mating. Daily exposure of the females was continued through the mating period and up to the weaning of their neonates, which were not exposed.

Twenty-four hours after birth, each pup was examined, sexed, and weighed. Each pup was reexamined and reweighed 4 days after birth. At this time, to equalize the nursing burden on each dam, each litter was reduced to no more than 10 pups/litter.[6,8,9] After weighing on day 21, two pups of each sex were randomly selected from each litter, were euthanized, and examined for gross external and visceral abnormalities. If no abnormalities were found, the remaining pups were assumed to be normal and were euthanized and discarded. If abnormalities were found among the first four, each remaining pup in the litter was euthanized and examined to determine the frequency of the abnormalities within the litter. The data from this study were analyzed using the Student's "t" test.

3. RESULTS

3.1 Fetal Toxicity and Teratogenicity.

The mean weight of the dams exposed to exhaust only was significantly lower than the mean weight for the controls on gestation days 6, 15, and 20. The same was true for the control versus the smoke groups on day 6 (Table 3). Analysis by the Student's "t" test showed no significant difference in the pregnancy rate, number of implant sites, mean live implants, mean implants per dam, or fetal body weights among the groups. A significantly higher number of in utero deaths occurred in the DF-2 smoke group (Table 4). During gross examinations of the fetuses, in the DF-2 smoke group, we found three fetuses with major malformations in one litter. One female exhibited exencephaly and was small, weighing 2.39 g. The second fetus (a male) exhibited clubbed feet, spina bifida, and weighed only 1.97 g (the average weight for the normal litter mates was 2.97 g), and the third fetus (a female) exhibited spina bifida. The first two fetuses were cleared and stained for skeletal examination. The first had a distorted cranium and short body. The second had divided cranial plates, cervical vetebra, and upper thoracic vertebra (Figure 1). The third fetus was placed in Bouin's Solution. When the fetus was examined viscerally, it showed signs of hemorrhaging around the olfactory bulbs, exhibited myeloschisis with distortion of the spinal cord, bilateral hydronephrosis and hydroureter, and schistocelia with evagination of intestine and fat. A fourth fetus in this litter was examined viscerally and had a diaphramatic hernia, and a fifth fetus had greatly distended ventricles with no

Table 3. DF-2 Teratology Maternal Body Weights

	Gestation Day	Means C/E*	SD C/E	DF	T	T-Table	Sig
Control vs. 60-min exhaust	6	263.17/247.85	16.65/16.44	36	2.85	2.03	+
	15	297.89/285.20	20.15/15.23	36	2.20	2.03	+
	20	358.55/340.95	21.74/22.05	36	2.47	2.03	+
	20	358.55/340.95	21.74/22.05	36	2.47	2.03	+
Control vs. 60-min smoke	6	263.17/249.90	16.65/18.77	36	2.29	2.03	+
	15	297.89/287.40	20.15/20.48	36	1.59	2.03	-
	20	358.55/352.99	21.74/24.46	36	0.74	2.03	-

LEGEND:
C = Control
E = Exposed
SD = standard deviation
DF = degrees of freedom
T = value of t
T-Table = table value of t
sig = significant

Table 4. Teratology Pregnancy Data

Conditions	Noise Control	Exhaust	DF-2 Smoke
Number Mated	20.0	20.0	20.0
Number Pregnant	18.0	20.0	20.0
% Pregnant	90.0	100.0	100.0
Total Implants	211.0	223.0	240.0
Live Implants	206.0	213.0	217.0
Dead Implants	5.0	10.0	23.0
% Dead Implants	2.37	4.48	9.58
Mean Implants per Pregnant Female	11.72 ± 3.51	11.10 ± 3.84	11.95 ± 2.52
Mean Live Implants per Pregnant Female	11.44 ± 3.43	10.65 ± 3.66	10.85 ± 2.62
Mean Dead Implants per Pregnant Female	0.28 ± 0.57	0.50 ± 0.83	1.15 ± 1.14
Mean Body Weights			
Males	3.60 ± 0.30 (108)	3.46 ± 0.35 (104)	3.62 ± 0.38 (102)
Females	3.40 ± 0.28 (98)	3.32 ± 0.34 (109)	3.46 ± 0.35 (115)
Combined	3.50 ± 0.31 (206)	3.39 ± 0.35 (213)	3.53 ± 0.37 (217)

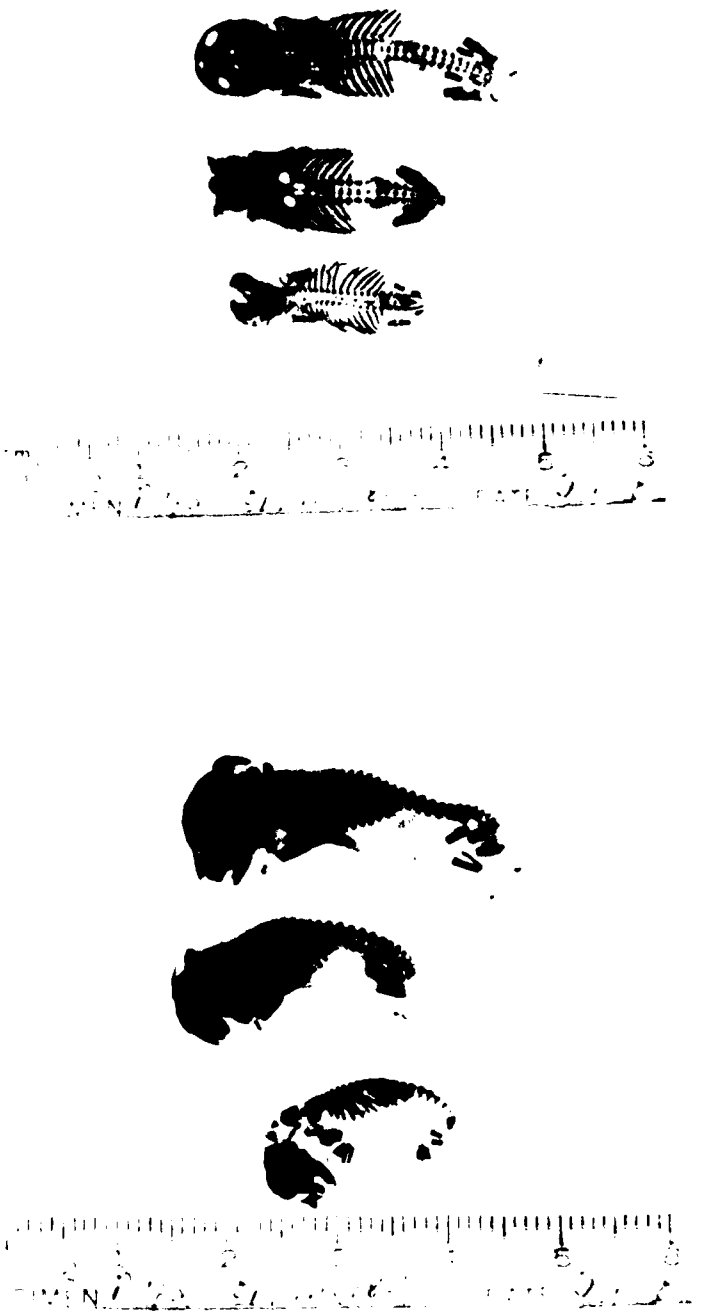

Figure 1. Comparison of Normal Rat Fetus with Two From a Dam Exposed to DF-2 Smoke. The top fetus is normal, the middle fetus has distorted cranium and short body, the bottom fetus has divided cranium, cervical vertebra, and upper thorax vertebra.

apparent cranial distortion. One fetus in the exhaust group had intestines extruding at the site of the umbilicus (Figure 2). No major abnormalities were seen in the noise control group.

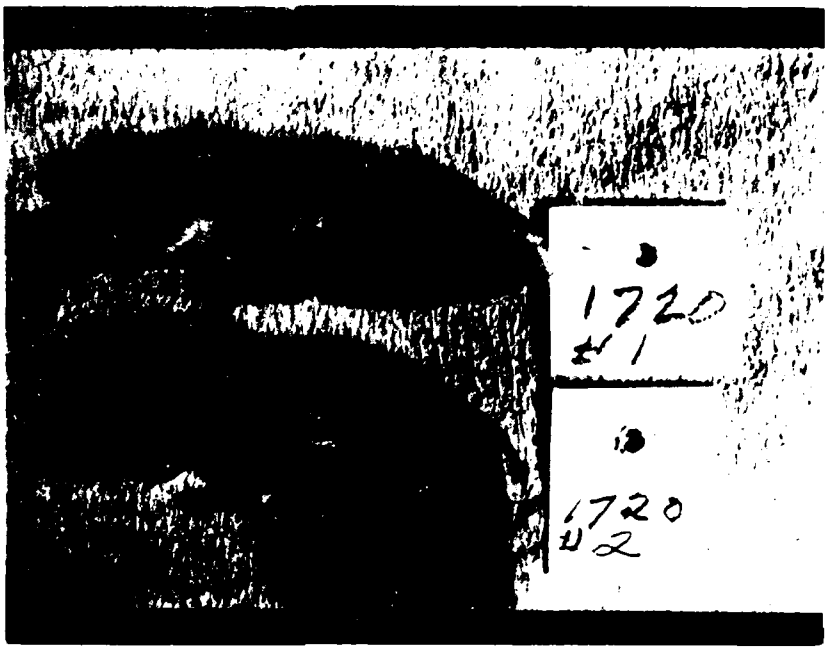

Figure 2. Comparison of a Normal Rat Fetus with One with Extruding Intestines from the Exhaust Study Group

There were a few visceral variations that were exhibited across groups. These variations included enlarged renal pelvises and abdominal hematomas. In the noise and exhaust groups, variations were seen involving the eyes and heart; however, these types of variations were not seen in the DF-2 smoke group.

Minor skeletal variations were also observed across groups; these variations included sites of retarded ossification and were concentrated in the vertebral column, the ribs, and the sternum (Table 5). The percentages of fetuses showing sites of low ossification follow:

Noise Control	Exhaust	DF-2 Smoke/Exhaust
64.55%	77.88%	81.03%

Table 5. Skeletal Variations Among Fetuses from Dams Exposed to Control Noise, Exhaust, or DF-2 Smoke During Organogenesis

	SEX OF FETUSES	NUMBER OF FETUSES	BILATERAL RUDIMENTARY RIB BUDS	RUDIMENTARY RIB BUD RIGHT	RUDIMENTARY RIB BUD LEFT	CLEFT VERTEBRAE, THORACIC	COMPLETELY DIVIDED VERTEBRAE, THORACIC	"DUMBBELL"-SHAPED VERTEBRAE, THORACIC	CLEFT STERNEBRAE	COMPLETELY DIVIDED STERNEBRAE	"DUMBBELL"-SHAPED STERNEBRAE	DEFICIENT OSSIFICATION STERNEBRAE &/XIPHOID PROCESS	IRREGULAR OSSIFICATION STERNEBRAE	NON-OSSIFIED STERNEBRAE	NON-OSSIFIED XIPHOID PROCESS
NOISE CONTROL	♀	48	0	3	0	3	1	2	5	0	10	14	1	10	2
	♂	60	3	2	2	1	1	1	11	6	3	15	4	5	1
EXHAUST	♀	56	2	3	1	0	1	9	7	1	10	25	1	13	4
	♂	56	2	1	4	0	0	4	9	0	17	25	1	2	2
DF 2 SMOKE	♀	60	1	2	4	3	2	14	7	2	12	25	0	13	4
	♂	54	2	3	3	0	1	5	5	1	16	24	0	9	3

3.2 Dominant Lethal Mutation Screen.

With the exception of one male in the 60-min exhaust group and one in the noise control group, all males successfully impregnated at least one of the available females in each of the two postexposure mating periods. The male in the noise control group failed to impregnate either female in the second week postexposure mating; the one male in the 60-min exhaust group failed to impregnate either female in either of the two postexposure mating periods (Table 6). Pregnancies were ascertained on the estimated 18th day of gestation (assuming conception occurred within the first 24 hr of cohabitation). The following recordings were calculated from the data obtained: mating index, corpora lutea index, implantation index, preimplantation loss index, fetal index, resorption index, nonviable (NVF) to viable (VF) fetus ratio, percentage of dams with one or more nonviable fetuses, and percentage of dams with two or more nonviable fetuses.

The number of dams with one or more resorptions (NVF) was significant using Chi-square analysis[10] in the second week matings for the 15-min exhaust group. None of the other parameters showed any significance for any other group (Tables 7 and 8). However, arc sinc transformation of resorption means followed by Student's "t" test showed no significant increase of resorptions in any treatment group (Table 9).

3.3 Reproduction in a Single Generation.

In the SG study, the mating, period of gestation, delivery, and care of neonates were of similar quality across groups. Student's "t" test showed no significant difference in the mean number of live births per group (Table 10). However, we determined that the average first day body weight of male pups in the 60-min exhaust group was significantly lower than the weight of the controls, and although the litters had been culled to a maximum of 10 pups each on the fourth day, the seventh day average body weight for female pups in the 60-min exhaust group was also significantly lower than the average seventh day body weight for the pups in the control group. By day 21, no significant difference in average body weight was apparent for any of the groups (Tables 11 and 12). However, a "t" test of the mean weight gain among the pups by day 21 showed that pups in the 60-min smoke group had a significantly lower weight gain from the first day (Table 13). Calculations of the viability and lactation indices showed no significant differences between the litters in the control and exposed groups (Table 14). Among the controls, necropsies of the 21-day-old pups showed one male and one female runt, four females with hydronephrosis, and one female with unilateral anophthalmia. In the 15-min exhaust group, there were one male and one female runt, one female whose paracardial sac contained fluid, two females with hydronephrosis, and one male with an underdeveloped testicle. In the 60-min exhaust group, one female had an unusually short body; two males and four females had hydronephrosis.

For the DF-2 smoke group 15-min exposure, one female had hydronephrosis. In the 60-min exposure group for the DF-2 smoke, there were one female runt, two males and four females with hydronephrosis, and one male with malformed eyelids on one eye.

Table 6. DLM Male Mating Record for DF-2 Smoke

Noise control			15-min exhaust			60-min exhaust			15-min exhaust			60-min exhaust		
Male No.	No. Dams Wk-1	Preg Wk-2	Male No.	No. dams Wk-1	preg Wk-2	Male No.	No. dams Wk-1	preg Wk-2	Male No.	No. dams Wk-1	preg Wk-2	Male No.	No. dams Wk-1	preg Wk-2
60	2	2	360	2	2	560	2	2	760	2	2	960	2	2
61	1	2	361	2	1	561	2	2	761	1	2	961	2	2
62	2	2	362	1	2	562	0	0	762	2	2	962	2	1
63	1	2	363	2	2	563	1	2	763	2	1	963	2	2
64	2	1	364	2	1	564	1	2	764	2	2	964	1	2
65	1	2	365	2	2	565	2	1	765	1	2	965	1	2
66	1	2	366	2	1	566	1	2	766	2	1	966	1	1
67	2	1	367	2	2	567	1	2	767	1	2	967	1	2
68	2	0	368	1	2	568	1	2	768	1	2	968	2	2
69	1	2	369	2	2	569	1	2	769	2	1	969	2	2

Table 7. Reproductive Data for Female Rats Mated to Male Rats Exposed to Noise, Exhaust, and DF-2 Smoke

	Week	Mated	No. Pregnant	M.I.[a]	C.L.I.	I.I.	P.I.L.I.	F.I.	R.I.	N.V.F./V.F.	N.V.F. $\geq$ 1	N.V.F. $\geq$ 2
NOISE CONTROL	1	20	15	75	(168) 11.20 ± 3.97	(158) 10.53 ± 4.52	(10) 0.67 ± 1.23	(153) 10.20 ± 4.47	(5) 0.33 ± 0.49	5/153	5/15	0/15
	2	20	16	80	(204) 12.75 ± 2.11	(162) 10.12 ± 4.08	(42) 2.63 ± 2.67	(149) 9.31 ± 4.03	(13) 0.81 ±2.01	13/149	3/16	2/16
15-MIN EXHAUST	1	20	18	90	(235) 13.06 ± 2.51	(199) 11.05 ± 4.19	(36) 2.08 ± 2.67	(190) 10.55 ± 4.08	(9) 0.50 ±0.78	9/190	3/18	3/18
	2	20	17	85	(219) 12.88 ± 2.20	(183) 10.76 ± 3.58	(36) 2.12 ± 3.02	(173) 10.18 ± 3.64	(10) 0.59 ±0.51	10/173	10/17*	0/17
60-MIN EXHAUST	1	19	12	63	(154) 12.83 ± 1.34	(140) 11.67 ± 1.87	(14) 1.17 ± 1.11	(131) 10.92 ± 2.02	(9) 0.75 ±1.21	9/131	3/12	2/12
	2	20	17	85	(226) 13.29 ± 2.93	(189) 11.12 ± 3.94	(37) 2.18 ± 1.94	(162) 10.29 ± 4.15	(14) 0.82 ±1.18	14/162	5/17	3/17

* = >55%

LEGEND:

M.I. (mating index) = $\frac{\text{total number of females pregnant}}{\text{total number of females mated}} \times 100$

C.L.I. (corpora lutea index) = $\frac{\text{total number of corpora lutea}}{\text{total number of pregnant females}}$

I.I. (implantation index) = $\frac{\text{total number of implantation sites}}{\text{total number of pregnant females}}$

P.I.L.I. (pre-implantation loss index) = $\frac{\text{total number of corpora lutea-total number of implantation sites}}{\text{total number of pregnant females}}$

F.I. (fetal index) = $\frac{\text{total number of viable fetuses}}{\text{total number of pregnant females}}$

R.I. (resorption index) = $\frac{\text{total number of (early plus late) deaths}}{\text{total number of pregnant females}}$

N.V.F./V.F. = $\frac{\text{total number of nonviable fetuses}}{\text{total number of viable fetuses}}$

N.V.F.$\geq$1 = $\frac{\text{total number of females with one or more nonviable fetuses}}{\text{total number of females with zero nonviable fetuses}}$

N.V.F.$\geq$2 = $\frac{\text{total number of females with two or more nonviable fetuses}}{\text{total number of females with one or zero nonviable fetuses}}$

Table 8. Reproductive Data for Female Rats Mated to Male Rats Exposed to Noise, Exhaust, and DF-2 Smoke

Numbers in Parentheses Indicate Total Numbers Per Category

	Week	Mated	# Pregnant	M.I.[a/]	C.L.I.	I.I.	P.I.L.I.	F.I.	R.I	N.V.F/V.F.	N.V.F.≥1	N.V.F.≥ 2
NOISE CONTROL	1	20	15	75	(168) 11.20 ±3.97	(158) 10.53 ± 4.52	(10) 0.67 ±1.23	(153) 10.20 ± 4.47	(5) 0.33 ±0.49	5/153	5/15	0/15
	2	20	16	80	(204) 12.75 ±2.11	(162) 10.12 ± 4.08	(42) 2.63 ±2.67	(149) 9.31 ± 4.03	(13) 0.81 ±2.01	13/149	3/16	2/16
[illegible] MIN SMOKE	1	20	16	80	(214) 13.38 ±1.36	(186) 11.62 ± 2.73	(28) 1.75 ±2.32	(175) 10.94 ± 3.42	(11) 0.69 ±0.79	11/175	5/16	3/16
	2	20	17	85	(213) 12.53 ±2.81	(182) 10.70 ± 3.08	(31) 1.82 ±2.32	(172) 10.12 ± 3.42	(10) 0.65 ±0.86	10/172	6/17	2/17
[illegible] MIN SMOKE	1	20	16	80	(207) 12.94 ± 1.81	(187) 11.69 ± 1.54	(20) 1.25 ±1.44	(176) 11.00 ± 2.22	(11) 0.69 ±1.19	11/176	6/16	2/16
	2	20	16	80	(244) 13.56 ±2.12	(218) 12.11 ± 1.74	(26) 1.46 ±2.09	(204) 11.33 ± 2.03	(14) 0.78 ±1.06	14/205	6/18	3/18

LEGEND: MI = Mating Index

C.L.I = Corpora Lentio Index

I.I. = Implantation Index

P.I.L.I. = Preimplantation Loss Index

F.I. = Fertility Index

R.I. = Resorption Index

N.V.F. = Nonviable Fetuses

V.F. = Viable Fetuses

Table 9. DF-2 DLM. Resorption data transformed using arc-sine, then the means are compared using Student's "t" tests

	Wk	Transformed Means C/E	SD C/E	DF	T	T-Table	Sig
Control vs. 15-min exhaust	1	15.97/14.92	15.26/10.09	31	0.24	2.04	-
	2	15.47/16.64	11.11/ 7.57	31	0.36	2.04	-
Control vs. 60-min exhaust	1	15.97/14.92	15.26/ 9.03	25	0.21	2.06	-
	2	15.47/19.84	11.11/16.04	31	0.90	2.04	-
Control vs. 15-min DF-2	1	15.97/15.90	15.26/ 8.99	29	0.02	2.045	-
	2	15.47/15.98	11.11/ 9.68	31	0.14	2.04	-
Control vs. 60-min DF-2	1	15.97/14.58	15.26/ 9.92	29	0.30	2.045	-
	2	15.47/15.46	11.11/ 8.69	32	0.001	2.04	-

LEGEND:
C = control
E = exposed
SD = standard deviation
DF = degrees of freedom
T = value of t
T-Table = table value of t
Sig = significant

Table 10. DF-2 Single Generation Live Births

	Mean C/E	SD C/E	DF	T	T-Table	Sig
Control vs. 15-min exhaust	11.30/10.47	2.41/3.58	31	0.84	2.04	-
Control vs. 15-min smoke	11.30/10.74	2.41/1.94	36	0.81	2.03	-
Control vs. 60-min exhaust	11.30/11.50	2.41/2.44	38	-0.26	2.02	-
Control vs. 60-min smoke	11.30/11.50	2.41/3.59	33	-0.21	2.04	-

LEGEND:
C = control
E = exposed
SD = standard deviation
DF = degrees of freedom
T = value of t
T-Table = table value of t
sig = significant

Table 11. Mean Body Weights and Standard Deviations for Single Generation Pups of Parents Exposed to Noise, Exhaust, and DF-2 Smoke

AGE OF PUPS	NOISE CONTROL						15-MIN EXHAUST						60-MIN EXHAUST					
	MALES			FEMALES			MALES			FEMALES			MALES			FEMALES		
Days	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
1	6.89	0.87	109	6.56	0.88	117	7.00	0.76	89	6.61	0.76	110	6.63 *	0.69	114	6.41	0.85	116
4	10.43	1.76	90	10.13	1.63	100	10.81	1.23	83	10.39	1.44	99	10.16	1.38	97	10.07	1.56	94
7	16.53	2.55	90	16.22	2.05	97	17.11	1.51	82	16.57	1.76	95	15.92	1.74	96	15.60 *	1.89	95
14	32.60	3.89	89	31.57	3.24	97	32.80	3.34	82	32.11	3.10	95	31.46	4.09	96	31.02	4.24	95
21	51.21	6.58	89	49.55	5.44	97	51.39	5.71	82	50.34	4.65	95	50.44	5.67	95	48.62	6.22	95

* $P < 0.05$ Student's "t" Test

LEGEND: SD = Standard Deviation

N = Number of Individuals

Table 12. Mean Body Weights and Standard Deviations for Single Generation Pups of Parents Exposed to Noise, Exhaust, and DF-2 Smoke

AGE OF PUPS	NOISE CONTROL						15-MIN SMOKE						60-MIN SMOKE					
	MALES			FEMALES			MALES			FEMALES			MALES			FEMALES		
Days	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
1	6.89	0.87	109	6.56	0.88	117	7.07	0.76	111	6.65	0.67	94	6.75	0.63	122	6.41	0.61	108
4	10.43	1.76	90	10.13	1.63	100	10.99	1.28	100	10.53	1.29	80	10.22	1.36	93	9.81	1.24	85
7	16.53	2.55	90	16.22	2.05	97	17.15	1.93	100	16.51	1.97	80	16.41	1.88	93	15.73	2.00	84
14	32.60	3.89	89	31.57	3.24	97	33.06	3.28	100	32.53	3.10	80	32.38	3.02	93	31.47	3.20	83
21	51.21	6.58	89	49.55	5.44	97	51.95	5.17	100	50.76	4.23	80	50.31	5.15	93	48.68	4.95	83

LEGEND: SD = Standard Deviations
N = Number of Individuals

Table 13. Students "t" Test of Weight Gain in Neonatal Rats from First to Fourth Weighings During Exposure to Exhaust or Smoke

Dose Group	N Value	Mean Gain	Degrees of freedom	T Value
Control	183	43.63	360	-1.53
15-min smoke	179	44.47		
Control	183	43.63	347	0.14
15-min exhaust	166	43.77		
Control	183	43.63	338	2.12*
60-min smoke	157	42.43		
Control	183	43.63	360	0.38
60-min exhaust	179	43.45		

*Significant at 95% confidence

Table 14. DF-2 Smoke Viability and Lactation Indices in a Single Generation

Index	Control	Exhaust		Smoke	
		Low	High	Low	High
Viability[a]	99.56	100.00	99.13	98.05	96.52
Lactation[b]	97.89	98.88	99.48	100.00	98.88

$$^{a}\text{Viability} = \frac{\text{Number of pups alive on day 4 (pre cull)}}{\text{Number of pups born alive}} \times 100$$

$$^{b}\text{Lactation} = \frac{\text{Number of pups alive on day 21}}{\text{Number of pups alive on day 4 (post cull)}} \times 100$$

4. DISCUSSION

The teratogenicity of a compound can be missed if the administered dose is embryocidal. The significantly higher number of dams in the exhaust group with two or more resorptions would alert one to the possibility that the exhaust is embryocidal. However, because there was no significant lowering of the number of births in the concurrent SG study, there is no strong support for such a contention in this study. The importance of a teratology study is manifested by the significant increase in the number of malformed fetuses. The five cases of fetal malformations seen in the 60-min DF-2 smoke group were significant, and because our records from past studies with this particular strain of rats showed no tendency toward spontaneous malformations of this type, we are reluctant to rule out the compound effect. On the other hand, the fact that these cases occurred in the same litter does not give a strong indication that DF-2 smoke was the causative factor. Additional tests with an increase in the exposure time might provide a better opportunity to discern any dose-response relationship.

The one other major anomaly found in the exhaust group was not observed in any past studies with this animal stock. When coupled with the cases already cited, this phenomenon might prompt one to question the effects of the exhaust as well.

The increase in minor skeletal variations in the DF-2 smoke group versus the noise control group cannot be ignored even though it is evidenced by the apparently normal development of the SG pups. Such deficiencies are probably overcome with continued fetal development. Apparently, in utero exposure to DF-2 smoke during organogenesis does cause retardation in fetal skeletal development.

While the dams from the second mating with males in the 15-min exhaust group showed significantly more dams with one or more resorptions, no other group showed this degree of effect, most particularly none of the dams mated to males exposed to DF-2 smoke. In addition, the lack of significantly lower live births in the SG study lessens any support for a true DL effect. Thus, DF-2 smoke probably does not induce DLM.

In the SG portion of the study, the significantly lower seventh day mean body weights of female pups in the 60-min exhaust group and the lower first day mean body weights for male pups in that group would make one more concerned about the exhaust alone rather than the DF-2 smoke mixed with the exhaust. DF-2 smoke had no effect on reproduction, including mating, fertility, gestation, delivery, lactation, or survival; the mean weight gain for the 60-min smoke group was significantly lower than for the controls.

5. CONCLUSIONS

There is no substantial evidence in this study that DF-2 smoke causes teratogenic or mutagenic responses in rat fetuses or rat sperm, respectively. There is also no substantial evidence that smoke has a detrimental effect on reproduction. However, smoke can reduce weight gain in rats. We acknowledge that this study only used the rat as the experimental model; therefore, the conclusions should not be extrapolated to other animals and definitely not to man.

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